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Pyrrolizidine alkaloids in *Senecio madagascariensis* from Australia and Hawaii and assessment of possible livestock poisoning

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Abstract

The alkaloid content of *Senecio madagascariensis* collected from Australia and Hawaii was examined. Alkaloids were identified from the above ground whole plant (stems, leaves and flowers) by GC/MS analysis and included: senecivernine, senecionine, integerrimine, senkirkine, mucronatinine, retrorsine, usaramine, otosenine, acetylsenkirkine, desacetyldoronine, florosenine and doronine. Plant material collected from the Hawaiian Islands was found to be identical in pyrrolizidine alkaloid content to that from a single composite collection made from northern New South Wales, Australia. Overall, no appreciable differences in alkaloid content were found between locations, whereas variation among individual plants was evident. The average total pyrrolizidine alkaloid content varied from a low of 217 μg/g to a high of 1990 μg/g (dry weight basis) among the locations. Based on comparable alkaloid content and documented pyrrolizidine alkaloidosis cases from Australia, *S. madagascariensis* may pose a significant risk to livestock grazing heavily infested ranges on the Hawaiian Islands.

Keywords: *Senecio madagascariensis*; Pyrrolizidine alkaloids; Livestock poisoning; Hepatotoxicity; Hawaii; Australia

1. Introduction

A relatively recent infestation of a plant commonly called ‘‘fireweed’’ (*Senecio madagascariensis* Poir.) among the Hawaiian Islands has become a serious weed problem and heightened the concern of possible poisoning of grazing livestock in the area (Motooka et al., 2004; Thorne et al., 2005). Fireweed was first observed on the island of Hawaii in the early 1980s and is suspected to have been introduced by contaminated groundcover seed imported from Australia. The plant is a native of Madagascar and South Africa and was found in the Hunter Valley of New South Wales,

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Australia as early as 1918 (Parsons and Cuthbertson, 1992). This plant has also migrated to South America and is currently found in Argentina and Colombia (Burgueño-Tapia et al., 2001).

Many *Senecio* spp. contain hepatotoxic pyrrolizidine alkaloids and if grazed over a period of time may induce irreversible liver damage. In Australia, a number of livestock poisonings have been associated with the grazing of *S. madagascariensis* from the central coastal areas of New South Wales and in southeastern Queensland (Walker and Kirkland, 1981; Seaman, 1987; Seaman and Walker, 1985; Seawright et al., 1991). It should be noted that the original reports of cattle intoxication from *Senecio lautus* were subsequently attributed to *S. madagascariensis* after reexamination of the voucher specimens by the National Herbarium of New South Wales (Noble et al., 1994).

Interest in the pyrrolizidine alkaloid content of *Senecio* species is obviously stimulated by the worldwide distribution of the genus and the fact that pyrrolizidine alkaloidosis is a serious problem for livestock production; occasional human poisonings occur as well (Stegelmeier et al., 1999). A current search of the literature does not reveal any detailed report on the pyrrolizidine alkaloid content of *S. madagascariensis*. From collections in Colombia, *S. madagascariensis* was reported to contain cacalolide sesquiterpenes some of which exhibit antihyperglycemic activity in mice (Burgueño-Tapia et al., 2001). We therefore report herein the pyrrolizidine alkaloid content of *S. madagascariensis* collected in Australia and the islands of Hawaii and Maui, as well as an assessment of the potential of this plant to cause livestock poisonings.

2. Materials and methods

2.1. Plant materials

A composite sample of the above ground plant material including stems, leaves and flowers of *S. madagascariensis* Poir. (Compositae) was collected by A. A. Seawright, June 2000, 20 km west of Kyogle, Upper Eden Ck Road, Black Horse Ck Pty. Ltd., New South Wales, Australia. Plant identification was confirmed by the Queensland Herbarium, Environmental Protection Agency (voucher #: AQ 499204).

From the Hawaiian Islands, 100 *S. madagascariensis* samples were collected from 10 different locations on the islands of Hawaii and Maui (five sites each island). Collected plants were in full flower stage of growth and above ground plant material was collected. GPS identified locations were obtained and recorded: Island of Hawaii, H-1 (N19.44.349, W155.52.518; elev. 1251 m), H-2 (N19.45.116, W155.51.978; elev. 1068 m), H-3 (N19.45.726, W155.51.931; elev. 946 m), H-4 (N19.46.917, W155.50.692; elev. 763 m), H-5 (N19.49.161, W155.36.684; elev. 1952 m); Island of Maui, M-1 (Makai; N20 50.029, W156 17.889; elev. 714 m), M-2 (Piiholo Mauka; N20 49.914, W156 17.809; elev. 743), M-3 (Olinda Makai; N20 49.943, W156 17.832; elev. 743 m), M-4 (Olinda Mauka; N20 49.830, W156 17.845; elev. 756 m), M-5 (Baltizar Bridge; N20 51.282, W156 18.580; elev. 514 m). At each location 10 individual plants were collected and allowed to dry at ambient temperature before shipping to the Poisonous Plant Research Laboratory for analysis.

2.2. Alkaloid extraction

Dry plant material was ground in a Cyclotec 1093 sample mill and a 200 mg aliquot was extracted using a variation of the method previously reported by Molyneux et al. (1979) with 4 mL $CHCl_3$ and 4 mL 1 N HCl for 1 h in a sealed 15 mL test tube by mechanical rotation. After extraction, samples were centrifuged and the upper aqueous acid layer removed with a Pasteur pipette to a second test tube. The remaining plant material and $CHCl_3$ solutions were then extracted a second time with 2 mL of 1 N HCl for 5–10 min, centrifuged and the acid extract combined with the first. Zinc dust (~100 mg) was added and the samples mixed for 3 h to reduce N-oxides. The samples were then centrifuged and decanted into a third test tube to which concentrated ammonium hydroxide (28%) was added drop-wise (Pasteur pipette) until the samples were alkaline (pH 9–10). The samples were then extracted twice with $CHCl_3$ (4 mL, 2 mL) for 5 min by mechanical rotation, centrifuged to aid layer separation, and then the $CHCl_3$ layer removed and filtered through anhydrous sodium sulfate into a clean 10 mL test tube. The combined $CHCl_3$ extracts were placed in a heated metal block (60 °C) and evaporated to dryness under a flow of nitrogen. The samples were stored at −20 °C until analysis.

2.3. GC–MS analysis

Samples were prepared for analysis by the addition of 0.5 mL of $CHCl_3$ containing 10 ppm methyl stearate as a quantitation standard. Samples (2 μL) were analyzed by GC/MS using a Finnigan MAT GCQ equipped with a split/splitless injector and a DB-5MS (30 m × 0.25 mm; J&W Scientific) column. Injection port temperature was 225 °C and column oven temperature program was 100 °C for 1 min; 100–200 °C at 20 °C/min; 200–300 °C at 10 °C/min. Electron impact ionization at 70 eV was used with an ion source temperature of 200 °C. Chemical ionization mass spectra were collected using methane as the reagent gas to confirm molecular ions. The detector scanned a mass range of *m/z* 60–650. Thirteen individual pyrrolizidine alkaloids were detected and from them the 10 most abundant were marked and peak areas measured. The concentration of individual alkaloids was measured from peak area ratios to the internal standard multiplied by the response factor determined from the injection of 10 ppm methyl stearate *versus* a 25 ppm riddelliine alkaloid standard.

2.4. Alkaloid identifications

Individual pyrrolizidine alkaloids were identified from available standard samples, retention indices (RI) calculated by linear extrapolation from RI values generated from known pyrrolizidine alkaloid standards and assigned RI numbers from the literature and their mass spectra (EI and methane CI) (Witte et al., 1993; El-Shazly et al., 1998; Asres et al., 2004; Pelser et al., 2005). See Table 1 for complete listing of identified compounds and mass spectrometric data.

2.5. Statistical analysis

Alkaloid data from the islands of Hawaii and Maui were compared for inter- and intra- island differences using analysis of variance (SAS, 2005). Island differences for each alkaloid were tested using sites nested within islands as the error term. Alkaloid concentrations at sites were examined in a separate analysis of variance for each island. When site differences were detected, the least significant difference procedure was used to determine which sites were different at $\alpha < 0.05$.

3. Results and discussion

3.1. Identification of alkaloids

A total of 13 pyrrolizidine alkaloids were detected in *S. madagascariensis* plant material after alkaloid extraction and analysis by GC/MS (Fig. 1A). The alkaloids were macrocyclic diesters of retronecine (**1–3, 5–7**) and otonecine (**4**, **8–12**) bases (Fig. 2). Alkaloids based on retronecine were present in the plant material almost entirely as N-oxides and were observed in the GC–MS analysis only after Zn reduction of the aqueous acid solution.

Positive identification of each alkaloid was made by either direct comparison with available standards or in reference to its mass spectrum and chromatographic retention indices (Table 1). The identification of mucronatinine (**5**) remains tentative at this time as no standard sample, mass spectrum or retention index was available. The existing literature is somewhat equivocal as to its exact chemical structure. Mucronatinine was originally isolated from *Crotalaria mucronata* and reported as shown for compound (**5**), being an isomer of usaramine with the *E* configuration of the exocyclic double bond (Bhacca and Sharma, 1968). However, other literature reports identify mucronatinine as being identical to usaramine (**7**) (Sawhney and Atal, 1970; Mattocks, 1986; Zalkow et al., 1988). The alkaloid in question, as detected in *S. madagascariensis,* has an RI of 2578 (DB-5) and elutes immediately before retrorsine and its mass spectrum was similar to that reported for usaramine (**7**).

One minor alkaloid (RI = 2493) remains unidentified. Based on its mass spectra (EI and CI) it has a molecular weight of 349 and the fragmentation pattern is indicative of an unsaturated diester of retronecine base.

The two chlorinated alkaloids (**10**, **12**) have been reported as secondary metabolites from a number of different *Senecio* plant species; however, to ensure that they were not artifacts of the extraction protocol a representative sample was extracted using 1% H_2SO_4 as a replacement for 1 N HCl in the acid/base extraction procedure. Secondly, a crude methanolic extract of the plant was analyzed directly by electrospray mass spectrometry (ESMS) eliminating the use

Table 1
Identification of pyrrolizidine alkaloids by GC–MS

Alkaloid	RI DB-5	CI–MS $[M+H]^+$	M^+	Other fragments (relative abundance)
Senecivernine (**1**)	2330	336	335(3)	291(6), 248(12), 246(11), 220(45), 202(14), 152(17), 136(35), 121(80), 120(83), 106(30), 94(100), 80(21).
Senecionine (**2**)[a]	2341	336	335(6)	246(43), 220(43), 218(17), 202(12), 178(9), 165(10), 152(12), 138(21), 136(57), 121(78), 120(93), 119(59), 109(20), 108(18), 106(27), 94(100), 80(22).
Integerrimine (**3**)[a]	2402	336	335(4)	291(7), 248(12), 246(9), 220(32), 138(17), 136(46), 122(21), 121(100), 120(75), 119(61), 109(22), 108(15), 106(32), 94(90), 80(20).
Unknown	2493	350	349(9)	349(9), 262(9), 244(11), 151(18), 138(20), 136(41), 123(25), 122(35), 121(43), 120(83), 119(55), 118(30), 108(18), 106(22), 94(100), 80(24).
Senkirkine (**4**)[a]	2530	366	365(3)	337(4), 321(7), 294(11), 266(18), 250(17), 222(24), 211(54), 168(38), 151(100), 139(19), 123(45), 110(36), 94(40), 81(29).
Mucronatinine (**5**)[b]	2578	352	351(5)	320(6), 292(5), 220(26), 218(10), 152(9), 138(15), 136(28), 122(22), 121(65), 120(100), 119(37), 106(23), 94(77), 80(15).
Retrorsine (**6**)[a]	2580	352	351(8)	320(5), 247(11), 246(51), 220(34), 218(18), 202(11), 178(12), 152(12), 138(21), 136(58), 122(26), 121(79), 120(100), 119(62), 118(24), 108(17), 106(23), 94(99), 80(22).
Usaramine (**7**)[a]	2640	352	351(5)	307(5), 248(6), 220(15), 148(20), 138(17), 136(44), 122(24), 121(100), 120(82), 119(58), 118(27), 108(14), 106(31), 94(85), 80(18).
Otosenine (**8**)[a]	2670	382	381(1)	381(1), 353(7), 308(6), 294(8), 282(6), 267(12), 266(69), 250(16), 168(81), 152(37), 151(100), 150(58), 125(24), 124(33), 123(68), 122(89), 110(41), 108(51), 96(37), 94(49).
Acetylsenkirkine (**9**)	2702	424	423(2)	364(5), 348(10), 321(12), 320(40), 303(30), 168(100), 166(36), 153(42), 150(26), 136(33), 122(34), 121(36), 110(41), 107(44), 95(25), 94(25).
Desacetyldoronine (**10**)	2737	418	417(1)	354(16), 340(13), 338(26), 336(28), 320(12), 292(21), 263(14), 254(18), 250(53), 238(14), 226(22), 168(100), 151(97), 150(61), 124(40), 123(89), 122(68), 110(40), 108(56), 96(35), 94(39), 82(33).
Florosenine (**11**)[a]	2825	424	423(2)	423(2), 408(4), 364(6), 363(6), 348(10), 336(36), 320(24), 319(67), 308(17), 304(100), 266(14), 264(10), 250(27), 238(13), 168(93), 166(28), 150(69), 148(36), 141(35), 122(57), 110(32), 94(26), 69(71).
Doronine (**12**)	2849	460	459(1)	459(1), 396(17), 380(8), 374(6), 372(15), 357(10), 355(28), 336(7), 320(16), 292(10), 238(7), 218(6), 177(18), 168(100), 150(41), 122(24), 94(11).

[a] Standards available for comparison. All other identified from RI and MS data.
[b] Tentative identification.

of any acids or chlorinated solvents altogether. The GC analyses of the two acid/base extracted samples were identical with detection of both doronine (**12**) and desacetyldoronine (**10**). In the ESMS analysis of a crude methanolic extract the corresponding protonated molecules were detected at $m/z = 418$ (MH^+ for desacetyldoronine) and $m/z = 460$ (MH^+ for doronine) and their identities confirmed from the MS/MS spectra comparison obtained on alkaloids after isolation using the acid/base procedure. These results confirm the alkaloids were present in the intact plant material and are true secondary metabolites of the plant.

3.2. Implication of alkaloid profiles

S. madagascariensis is native to Madagascar and southern Africa and although the means of introduction is unknown it was first observed in Australia in 1918; genetic analysis has shown that the African and Australian populations are closely related (Radford et al., 2000). The plant is now naturalized in coastal New South Wales and southern Queensland and has been declared a noxious weed (Queensland Government, 2005); it is commonly known as fireweed due to its propensity to invade bushland after fires. It is a major economic concern because of poisoning of cattle and horses, with signs of poisonings characteristic of those produced by hepatotoxic pyrrolizidine alkaloids typically occurring in many *Senecio* species (Mattocks, 1986).

In the Hawaiian Islands *S. madagascariensis* was first discovered in the early 1980s at the northernmost tip of the island of Hawaii and introduction is suspected to have been through contaminated groundcover seed from Australia

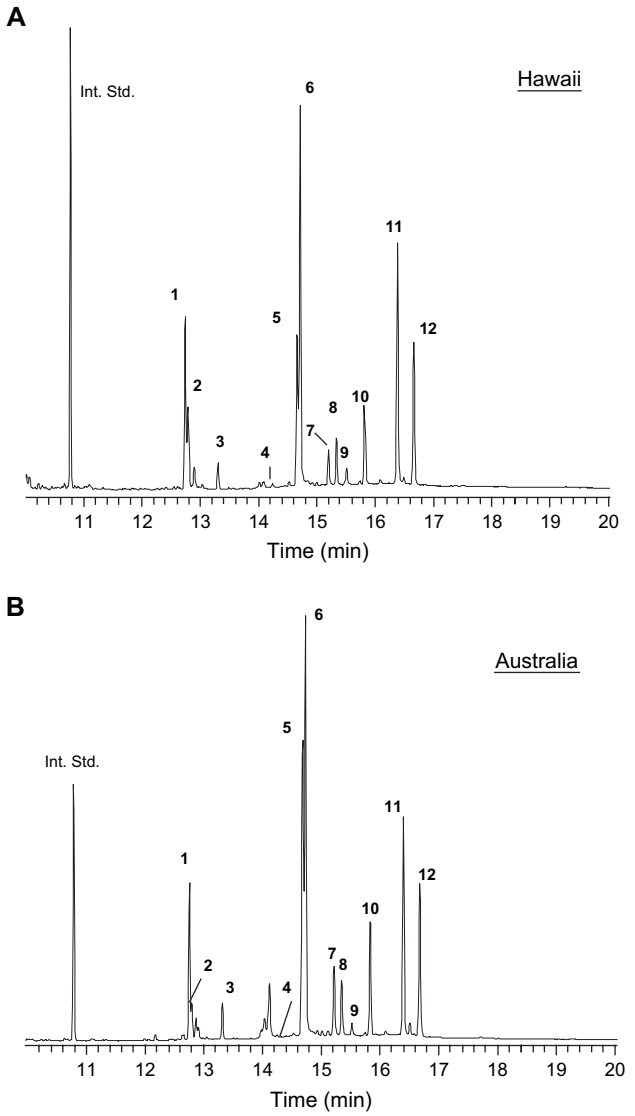

Fig. 1. Total ion chromatograms from GC/MS analysis of pyrrolizidine alkaloids from samples collected on Hawaii (A) and in Australia (B). Alkaloids identified include: senecivernine (**1**), senecionine (**2**), integerrimine (**3**), senkirkine (**4**), mucronatinine (**5**), retrorsine (**6**), usaramine (**7**), otosenine (**8**), acetylsenkirkine (**9**), desacetyldoronine (**10**), florosenine (**11**) and doronine (**12**).

(Starr et al., 1999; Motooka et al., 2004). It is now widespread on Hawaii and has since migrated northward to the adjacent island of Maui. The first signs of the weed on Maui were up-country on the north face of Haleakala (mountain) along the roadways. The general consensus is that the seed was introduced on Maui through groundcover seed, just as on Hawaii. A single infestation on Kauai at the northwestern end of the archipelago, known to have occurred with the application of groundcover seed along the highway, has fortunately been eradicated. It is possible that the infestation on Maui has been enhanced by the narrowness of the ʼAlenuihāhā Channel, a matter of only 30 miles, and consequent dispersion from prevailing easterly trade winds. The plant is now very widespread in the area around Waimea (Kamuela) the primary cattle-ranching area of Hawaii and can be observed in flower and seed stages there at all times of the year (Fig. 3). The potential for livestock poisoning in this area is therefore extremely high.

A general comparison of the alkaloids found in *S. madagascariensis* from the Hawaiian Islands and those from an Australian collection showed the two collections to have essentially the same alkaloids (Fig. 1B). The similarity of the alkaloid profiles of plants from Hawaii and Australia supports the suggestion that the Hawaiian plants may have originated in Australia.

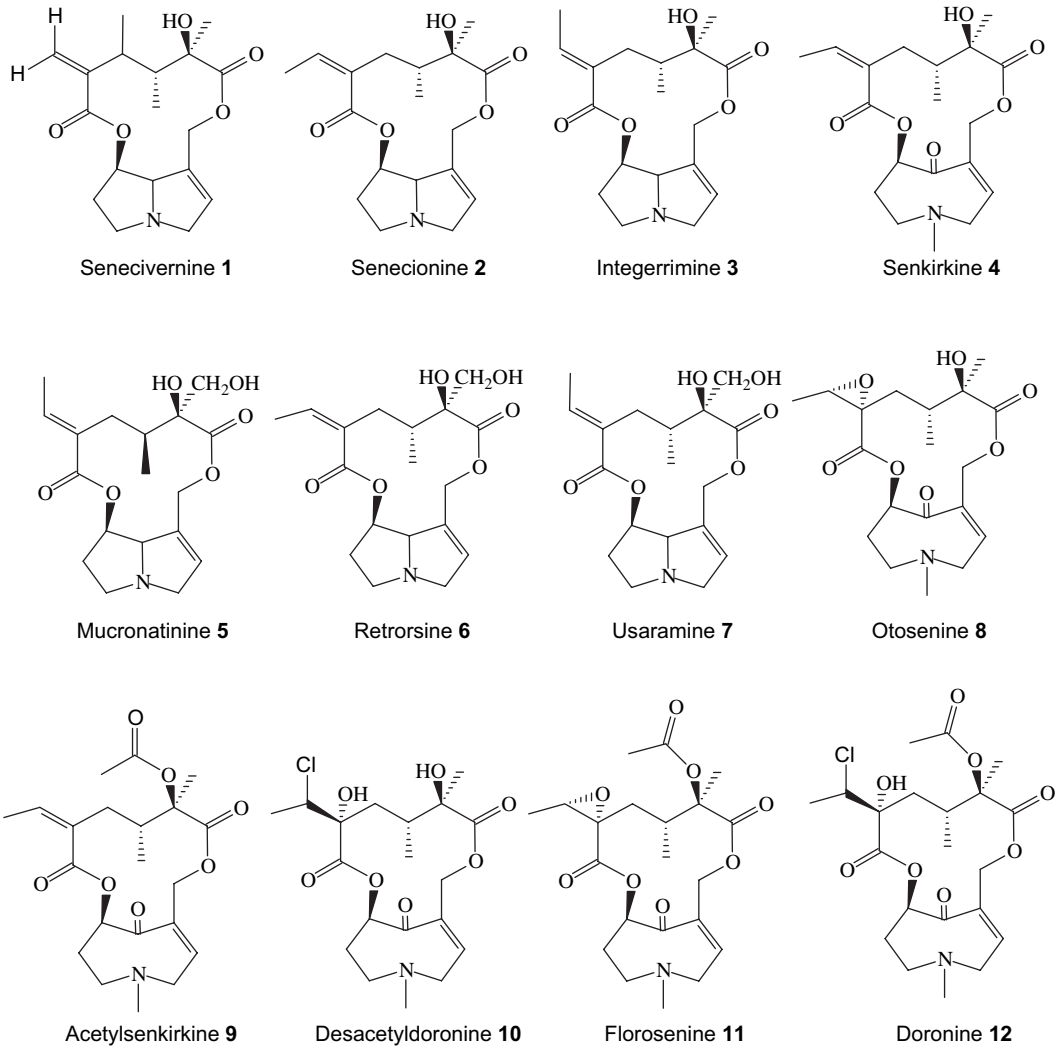

Fig. 2. Pyrrolizidine alkaloids isolated from *Senecio madagascariensis* from the Hawaiian Islands and Australia.

After a large number of individual plants had been collected from five locations each on the islands of Hawaii and Maui, a more detailed examination of the alkaloids was made. The collection sites were all in open rangeland areas that varied somewhat in elevation and soil type. The alkaloid content was highly variable among the individual samples both within and among locations. The average total pyrrolizidine alkaloid content among locations varied as much as a factor of 10 (220–2000 μg/g, dry weight basis total alkaloid) (Table 2). Variability within a given location was extremely large, and some individual plants contained essentially no pyrrolizidine alkaloids compared to levels in excess of 7000 μg/g (0.7% dry weight) for others. There was no statistical difference in alkaloid levels from samples collected on Hawaii *versus* Maui. However, the likelihood of finding a significant difference between locations was extremely low given the degree of individual sample variation among plants within a single location. Based on location averages, seven populations contained alkaloid levels below 1000 μg/g (480 ± 140 μg/g total pyrrolizidine alkaloid) and three populations contained alkaloid levels at levels greater than 1500 μg/g (1800 ± 260 μg/g total pyrrolizidine alkaloid). The difference between the two groups results from levels of the *seco* alkaloids (**8**–**12**), although senecivernine (**1**) was found to be significantly higher in the three latter locations as well.

The ecological factors that might drive plants from locations H-2, M-2 and M-3 to produce much greater quantities of alkaloid are unknown. However, studies of pyrrolizidine alkaloids in other *Senecio* species have shown analogous

Fig. 3. Horses grazing a pasture heavily infested with *Senecio madagascariensis* on the island of Hawaii.

variations (Johnson et al., 1985) and exceptionally high contents can be attained on occasion (Molyneux and Johnson, 1984).

The macrocyclic diester alkaloids identified in *S. madagascariensis* are of the most toxic pyrrolizidine alkaloid types (Mattocks, 1986). Alkaloid levels are similar to those reported for total pyrrolizidine alkaloid content in many *Senecio* spp. For example, *Senecio jacobaea* (tansy ragwort) was reported to contain 0.09–0.18% total pyrrolizidine alkaloid content in samples taken from the northwestern US where this plant has been responsible for numerous livestock losses (Molyneux et al., 1979). The level of pyrrolizidine alkaloid from the Australian sample was similar at 0.14%, and there are several documented cases of poisoning in Australian livestock from *S. madagascariensis* (Walker and Kirkland, 1981; Seaman, 1987; Seaman and Walker, 1985; Seawright et al., 1991). As such, those plants found in Hawaii should be considered toxic. Cattle grazing heavily infested pastures on the Hawaiian Islands would be at risk and horses, which are highly susceptible to pyrrolizidine alkaloid-induced hepatotoxicity (Mattocks, 1986; Stegelmeier et al., 1996), would be even more so. Typically, pyrrolizidine alkaloid-containing plants such as *Senecio* spp are not very palatable and not readily grazed and thus its abundance in relation to other available forage would be a major factor in establishing the possibility of poisoning under grazing conditions (Pfister et al., 2001). Poisoning might also occur from *S. madagascariensis* contamination in harvested feed supplies and thus crop lands in areas of known fireweed growth should be monitored for infestation before harvesting and processing. It is unlikely

Table 2
Measured levels (ppm) of the 10 most prominent pyrrolizidine alkaloids in *S. madagascariensis* from Hawaii Island collections

Location	**1**	**2**	**3**	**5**	**6**	**7**	**8**	**10**	**11**	**12**	Total PA
H-1	43 ± 37	32 ± 32	11 ± 8	56 ± 44	115 ± 82	21 ± 22	61 ± 97	55 ± 67	167 ± 244	75 ± 105	637 ± 497
H-2	**29 ± 40**	**19 ± 19**	**7 ± 17**	**31 ± 26**	**54 ± 72**	**3 ± 10**	**264 ± 164**	**117 ± 82**	**679 ± 467**	**306 ± 230**	**1508 ± 1035**
H-3	14 ± 9	14 ± 12	7 ± 6	43 ± 42	70 ± 40	16 ± 17	55 ± 78	38 ± 60	121 ± 127	76 ± 111	454 ± 308
H-4	5 ± 5	9 ± 8	6 ± 5	2 ± 7	96 ± 130	4 ± 12	36 ± 33	24 ± 21	193 ± 166	60 ± 43	436 ± 245
H-5	53 ± 45	15 ± 12	10 ± 7	31 ± 30	46 ± 29	4 ± 18	71 ± 107	92 ± 92	153 ± 165	123 ± 137	597 ± 436
M-1	38 ± 32	22 ± 31	9 ± 12	40 ± 39	49 ± 52	3 ± 5	28 ± 24	52 ± 46	124 ± 69	129 ± 93	494 ± 285
M-2	**218 ± 309**	**65 ± 88**	**17 ± 28**	**55 ± 79**	**71 ± 117**	**0**	**129 ± 154**	**228 ± 323**	**672 ± 834**	**439 ± 625**	**1894 ± 2055**
M-3	**95 ± 137**	**41 ± 35**	**24 ± 26**	**63 ± 73**	**111 ± 101**	**28 ± 90**	**108 ± 131**	**291 ± 317**	**648 ± 1226**	**580 ± 862**	**1990 ± 2603**
M-4	23 ± 27	12 ± 12	1 ± 4	16 ± 17	15 ± 16	0	42 ± 74	78 ± 135	162 ± 233	165 ± 524	513 ± 727
M-5	21 ± 34	16 ± 19	3 ± 6	29 ± 44	23 ± 26	0	12 ± 17	35 ± 70	35 ± 49	43 ± 58	217 ± 181

that *S. madagascariensis* can now be eliminated from the Hawaiian Islands and thus it is important that it be aggressively managed to reduce spreading and infestation of productive rangelands and possible poisoning of domestic livestock (Thorne et al., 2005).

3.3. Ecological significance

The endemic flora of the Hawaiian archipelago evolved in extreme isolation. Since the arrival of man, the islands have been inundated by an ever-increasing number of alien plant and animal introductions, many of which have displaced the native species. *S. madagascariensis* is one of the most recent examples, having been introduced within the last 25 years. It is believed to have not come directly from its native source in Africa but indirectly via Australia. The problems associated with this plant in poisoning of livestock in Australia have been well established since early in the last century. Fireweed is highly adaptable and can invade and survive in a great variety of conditions although frost in cooler areas of Australia may limit its spread (Sindel and Michael, 1992); such conditions are unlikely to be encountered in Hawaii except at elevations above those at which livestock are grazed. It is now impossible to eradicate fireweed from Hawaii and Maui but through integrated weed management strategies (Thorne et al., 2005) it is possible to prevent its spread to the other islands. More importantly, it should be recognized that *S. madagascariensis* is but one example of the large number of hepatotoxic *Senecio* species that could be introduced to Hawaii and that it is essential to be aware of the potential for such invasion either from primary or secondary sources.

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